conserved in vertebrates and (2) Golgi outposts are present in vertebrate neuron dendrites (Horton et al., 2005). Many other exciting questions remain to be addressed. Is the extent of Golgi-associated acentrosomal MT nucleation different in neuronal subtypes characterized by significantly different dendritic complexity, such as hippocampal neurons versus Purkinje cells? Is this process of acentrosomal MT nucleation used in other large, highly polarized cell types in the developing brain, such as dividing radial glial progenitors? What are the molecular mechanisms regulating the position, number and activity of Golgi-outpost acentrosomal MT nucleation sites in dendrites? Without any doubt, future studies will tackle the questions raised by these exciting new results.

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Dystroglycan Adds More Sugars to the Midline Cocktail

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In this issue of *Neuron*, Wright et al. (2012) identify two novel mediators of α-dystroglycan glycosylation in mouse and unravel a novel function of glycosylated dystroglycan in axon guidance by providing evidence for direct binding of α-DG to the midline chemorepellent Slit2.

The addition of glycan chains is a key step during the biosynthesis of many extracellular proteins, membrane bound receptors, and lipids. The structural diversity of these sugar polymers, further expanded by addition of sulfate, phosphate, and acetyl groups, is tremendous, possibly exceeding that of proteins (Ohtsubo and Marth, 2006). An increasing number of human diseases have been found to be caused by mutations in genes encoding glycosyltransferases and glycosidases (so-called congenital disorders of glycosylation or CDG; Freeze et al., 2012). In most cases, the development of the nervous system is affected (Freeze et al., 2012), such as in dystroglycanopathies, which are all linked to abnormal glycosylation of α-dystroglycan (α-DG).

Dystroglycan is a transmembrane protein expressed in various cell types that binds to laminin, a key component of the extracellular matrix (Hohenester and Yurchenco, 2012). The dystroglycan complex has thus been established as a crucial mediator of communication between factors of the extracellular matrix. The biosynthesis pathway of dystroglycan entails intracellular posttranslational proteolytic processing of a propeptide derived from a single mRNA, creating the α and β subunit of the mature dystroglycan (Hohenester and Yurchenco, 2012). Interestingly, following this initial cleavage, the two subunits reassemble noncovalently upon reaching the plasma membrane. The β-dystroglycan spans the plasma membrane, thus mediating intracellular signaling processes, while the α-dystroglycan is responsible for extracellular binding of ligands.

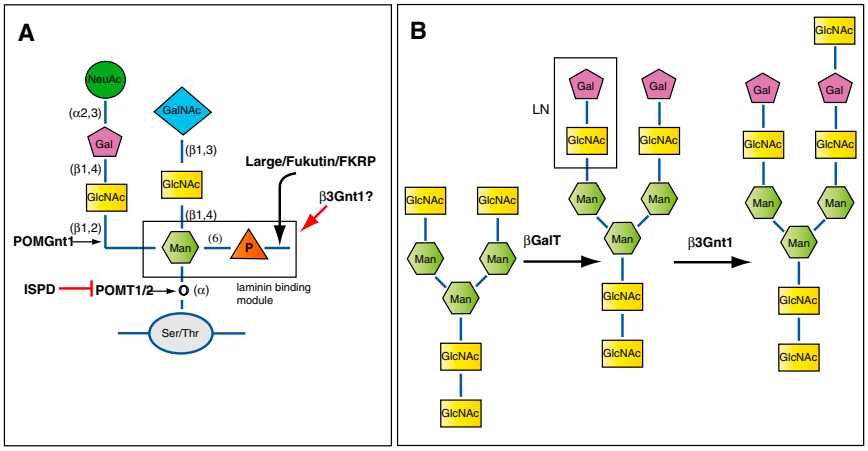

Figure 1. O-Glycosylation of α-Dystroglycan

(A) The posttranslational modification of α-Dystroglycan starts with the O-mannosylation of Ser/Thr residues by POMT1/POMT2. POMT1,2 and POMGnT1 furthermore catalyze the biosynthesis of an O-mannosyl-tetrasaccharide (NeuNAc-α-2,3-Gal-β-1,4-GlcNAc-β-1,2-Man) that has been shown to be a major constituent of muscle and brain α-dystroglycan. This glycosidic residue is then further branched and modified by phosphorylation on the mannitol subunit and subsequent postphosphoryl-glycosidations required for laminin binding. These reactions are catalyzed by dystroglycanopathy-related glycosyltransferases such as LARGE, Fukutin and FKRP. LARGE activity results in repeating units of [-3-xylose-α1, 3-glucuronic acid-β1-] by xylosyltransferase and glucuronyltransferase activities (not shown), which produce the postphosphoryl-carbohydrate chain. The exact function of ISPD and B3Gnt1 in α-DG glycosylation remains to be determined.

(B) β3GnT1 was initially identified by its function in the synthesis of Poly-N-Acetyllactosamine by transferring a donor UDP-N-Acetylglucosamine (GlcNAc) to a Galactose (Gal) acceptor moiety creating a β-1, 3-glycosidic linkage.

Abbreviations: Man, Mannose; GalNAc, N-Acetylgalactosamine; NeuAc, N-acetylneuraminic acid.

Glyco-epitopes on α-dystroglycan are recognized by Laminin, which through its polymerization functions as the key component in basement membrane assembly during embryogenesis (Hohenester and Yurchenco, 2012). To date, eight glycosyltransferases involved in the glycosylation of α-DG were identified through genetic mapping in the dystroglycanopathy patients (Freeze et al., 2012; Figure 1). The development of mouse models of dystroglycanopathies has proven difficult, and the dystroglycan conditional knockout *Pomgnt1* and *Large*^{myd} mice are the only existing models (Waite et al., 2012).

Here, Wright et al. (2012) performed an ENU (N-Ethyl-N-nitrosurea) mutagenesis screen in mouse to identify novel genes controlling axon guidance and describe two mutants exhibiting severe and axon pathfinding defects in the embryonic hindbrain and spinal cord. The mutated genes encode ISPD (isoprenoid synthase domain containing) and B3Gnt1 (β-1,3-N-Acetyl-glucosaminyltransferase), two enzymes previously linked to protein glycosylation. In prokaryotes and plants, ISPD is a nucleotidyl transferase which functions in isoprenoid precursor synthesis, a pathway that is substituted by the mevalonate axis in mammals. B3Gnt1 belongs to a family of eight glycosyltransferases (BGnt1–8) that are structurally related to β-1,3-galactosyltransferases and differ in substrate specificity and in vivo functions (Henion et al., 2012). B3Gnts catalyze the transfer of a donor UDP-N-Acetyl-glucosamine to a Galactose acceptor moiety creating a β-1,3-glycosidic linkage (Figure 1).

How do B3Gnt1 and ISPD influence axon guidance? Recently, it has been shown that human ISPD is critical for initiation of the glycosylation cascade, since in the absence of ISPD, the serine/threonine-O-mannosylation of α-DG in the endoplasmic reticulum and subsequent glycosylation events are severely reduced (Roscioli et al., 2012; Willer et al., 2012; Figure 1A). Accordingly, the authors found that α-DG glycosylation is strongly diminished in the mouse *ISPD* mutant. Interestingly, this is also the case in the *B3Gnt1* mutant. As expected, in both mutants, laminin binding to α-DG is abrogated. Although the ISPD mutants die at birth, the authors combined two different *B3Gnt1* mutant alleles to generate mice that survive for several weeks and develop many of the classic features of dystroglycanopathies, such as muscular dystrophy and neuronal radial migration defects in cortex, hippocampus and cerebellum.

To confirm that the axon guidance deficits observed in *ISPD* and *B3GnT1* mutants were linked to α-DG, Wright et al. (2012), in this issue of *Neuron*, used a DG conditional knockout line to selectively inactivate DG in the epiblast. This showed that axons also failed to extend properly in the hindbrain and spinal cord. Previous studies had linked α-DG and neuronal migration in mammals, but this is the first evidence that it also plays a role in axon guidance. However, the biggest surprise was still to come, when the authors found that the guidance of spinal cord commissural axons was severely perturbed in *ISPD*, *B3Gnt1* and *a-DG*, mutants. In normal embryos, most commissural axons turn rostrally after crossing the ventral midline (floor plate), whereas in the three mutants, these axons either fail to cross or grow randomly after crossing (Figure 2A). Although midline crossing defects have been detected in many knockout mice with targeted deletion of genes encoding axon guidance receptors and ligands (Chédotal, 2011), the authors showed that *Robo1/Robo2* double knockouts most faithfully recapitulate commissural axons phenotypes found in α-DG, *ISPD*, and *B3Gnt1* mutants. They went on to show that α-DG is highly expressed in floor plate, as is the case for laminin and the Robo ligands Slit1–3 (Brose et al., 1999), suggesting that α-DG and its glycan chains might bind and stabilize Slits at the midline. Worthy of note, the fasciculation of dorsal root ganglion (DRG) axons in the dorsal funiculus is perturbed in *B3Gnt1* and *ISPD* mutants. As previous studies showed that Slit2 is a branching factor for DRG axons (Wang et al., 1999), it suggests that glycosylated α-DG might control Slit localization or function outside the ventral midline.

Slits are large secreted proteins that act at the ventral midline as repulsive

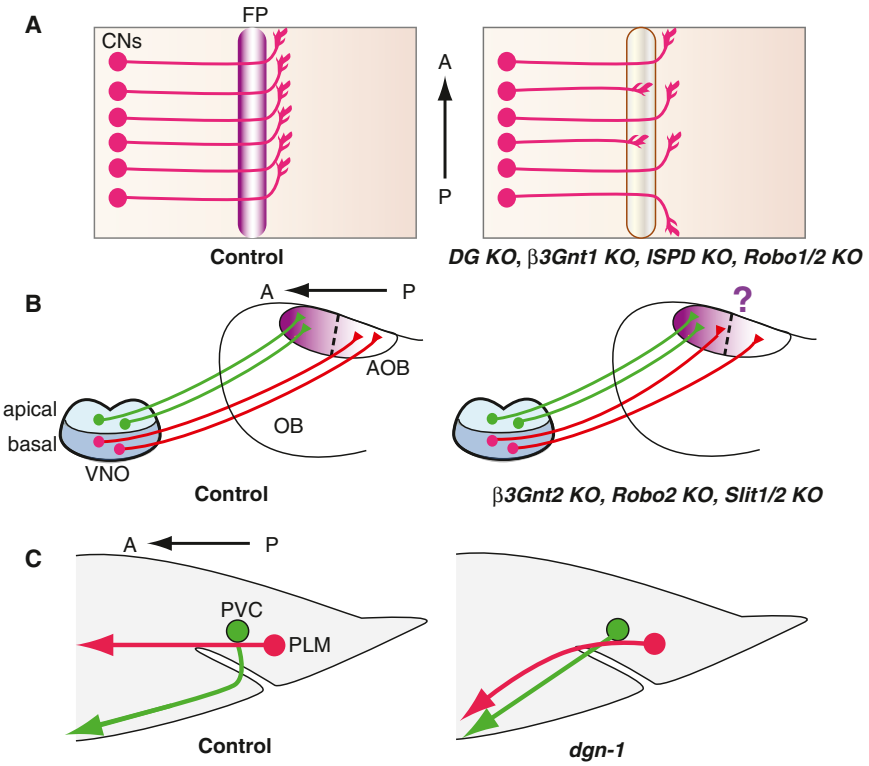

Figure 2. Axon Guidance Defects in Dystroglycan and Slit/Robo Mutants

(A) Spinal cord commissural neurons (CN) project axons toward the floor plate (FP) at the ventral midline where Slits are highly expressed (purple). After crossing most CN axons grow along the FP in a rostral direction. In *α-DG*, *ISPD*, and *B3Gnt1* mutants and *Robo1/Robo2* double knockouts, CN axons project ventrally but they either stall at the FP or project after crossing in a caudal or rostral direction. In *α-DG*, *ISPD*, and *B3Gnt1* mutants, Slits are not detected at the FP. The anterior (A) posterior (P) axis is indicated in all panels.

(B) Robo2$^+$ sensory neurons in the basal part of the vomeronasal organ (VNO) project to the posterior AOB. By contrast, in B3Gnt2 knockouts, some basal VNO axons also project to the anterior AO. In the AOB of control mice, Slit1 and Slit2 are expressed in a high-anterior to low-posterior gradient. It is not known if the gradient is maintained in B3Gnt2 KO.

(C) Premature ventral extension of two classes of axons in the *C. elegans* dystroglycan mutant *dg-1*.

guidance cues for ipsilaterally projecting axons and postcrossing commissural axons (Brose et al., 1999; Chédotal, 2011). Two Slit2 fragments can be purified from mammalian brain extracts (Nguyen Ba-Charvet et al., 2001; Wang et al., 1999): full-length Slit2 and a shorter N-terminal fragment (Slit2-N). Both Slit2 and Slit2-N bind to Robo receptors (Hohenester, 2008; Figure 3). Proteolytic processing of Slit2 generates a shorter C-terminal fragment (Slit2-C) which is unable to bind to Robo. Slit2-C function is unknown but it binds to heparan sulfate proteoglycans (HSPGs), another class of glycoproteins (Hohenester, 2008). HSPGs are also key components of the Slit/Robo binding domain and are thought to stabilize the interaction between the ligand and its receptor (Figure 3). As Slit2-C contains a laminin-G module found in all proteins known to bind to α-DG, Wright et al. (2012) next tested the ability of Slit2-C to bind α-DG. They found that Slit2-C binds to α-DG in a calcium-dependent manner, and Slit2-C also binds to floor plate in control mice but not in *B3Gnt1* mutant mice. Previous studies have shown that only Slit2-N and full length Slit2 mediate axon repulsion (Nguyen Ba-Charvet et al., 2001). Therefore, it will be important to show that full-length Slit2, in addition to Slit2-C, binds to α-DG.

A Robo-ectodomain (which binds all Slits in vitro) was then use to localize Slit proteins on spinal cord sections. As expected, a strong Robo binding was observed at the floor plate, confirming that this is the region with the highest levels of Slits in the developing spinal cord. Quite remarkably, Robo binding was lost in *B3Gnt1* mutant floor plate. These data strongly suggest that glycosylated α-DG, is orchestrating the distribution of Slit ligands in the extracellular matrix at the midline.

Intriguingly, genetic and biochemical evidence support a role for B3Gnt2, which is closely related to B3Gnt1, in axon guidance in sensory systems. In the mouse accessory olfactory system, sensory neurons in the basal vomeronasal organ (VNO) project to the caudal half of the accessory olfactory bulb (AOB; Figure 2B). In the AOB, Slit1 and Slit2 are expressed in a high-anterior to low-posterior gradient (Prince et al., 2009). In *Robo2* and in *Slit1* knockouts, some basal VNO axons invade the anterior AOB (Figure 2B), a phenotype recently described in the *B3Gnt2* knockout (Henion et al., 2012). It is possible that BGNT2 acts to shape the Slit gradient in the AOB or modulate Slit interaction with Robo2 receptors on VNO axons, and this phenotype will need to be characterized more closely.

These exciting results raise many important questions. Are Slits the only midline axon guidance proteins binding to α-DG? Recent work has demonstrated that DGN-1, the *C. elegans* homolog of α-DG, is required for appropriate development of the lumbar commissure (Johnson and Kramer, 2012; Figure 3C). Interestingly, in this system, genetic evidence suggests that the α-DG pathway is not only linked to Slit but also to UNC-6/netrin-1. These data support a role for dystroglycans in axon guidance but also suggest that netrin-1 localization might be perturbed in the *α-DG* and *B3GNT1* mutants. Further analysis will be required to determine if other Slit responsive axons are misguided in *α-DG/B3GNT1/ISPD* mutants. Interestingly, hindbrain pontine neurons, which are commissural, express Robo receptors, α-DG, Large and Fukutin, and in all the corresponding mutants (as in WWS patients) pontine neurons do not migrate properly toward the floor plate (see references in Waite et al., 2012).

Undoubtedly, this exciting study opens new perspectives in the axon guidance

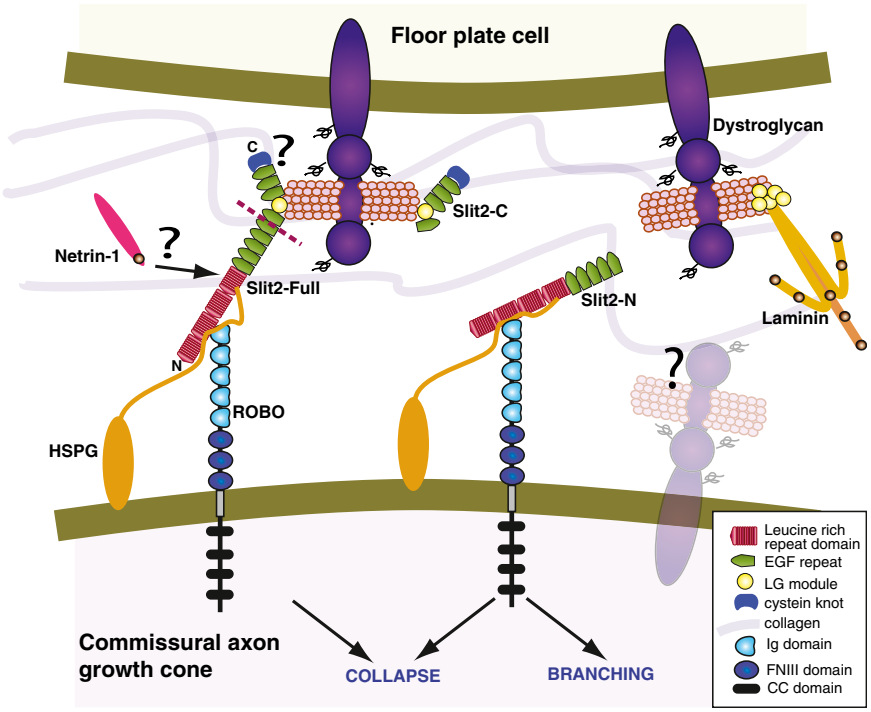

Figure 3. Dystroglycan in Axon Guidance
Dystroglycan on floor plate cells binds to laminin in the ECM and to the laminin-G module of Slit2-C thus controlling its distribution and presentation. Under the stabilizing action of HSPGs, full-length Slit2 and Slit-N D2 domain binds to the first immunoglobulin domain of the Robo receptors at the surface of commissural axons, inducing growth cone collapse or branching.

field and beyond, as Slit/Robo signaling regulate cell-cell interaction in many developing organs and in tumor cells and similarly, many of the B3gnt enzymes have also been shown to play a crucial role in tumorigenesis in many different cancers.

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